Naturwissenschaften (2012) 99:687–694
DOI 10.1007/s00114-012-0950-0

ORIGINAL PAPER

Different uses of plant semiochemicals in host location strategies of the two tachinid parasitoids

**Ryoko T. Ichiki · Giang T. T. Ho · Eric Wajnberg ·
Yooichi Kainoh · Jun Tabata · Satoshi Nakamura**

Received: 31 May 2012 / Revised: 9 July 2012 / Accepted: 11 July 2012 /

Abstract Some members of the family Tachinidae (Insecta: Diptera) deposit numerous very small eggs, termed "microtype" eggs, on the food plants of their caterpillar hosts. Parasitization is successful only when the hosts ingest these eggs. To increase the chance of hosts encountering the eggs, microtype tachinid parasitoids have to choose a suitable plant that harbors hosts and lay their eggs near the hosts. In their host location process, semiochemicals emitted by host-infested plants offer the tachinids a reliable cue. We investigated the behavioral responses of two microtype tachinid parasitoids, *Pales pavida* and *Zenillia dolosa*, to maize plants infested with their caterpillar host, *Mythimna separata*, in a wind tunnel. *P. pavida* females showed a significantly higher rate of landing on caterpillar-infested plants than on mechanically wounded or intact plants, whereas *Z. dolosa* landed on both the caterpillar-infested and mechanically wounded plants at significantly higher rates than on intact plants. We also examined which part of a caterpillar-infested maize leaf induces oviposition. *P. pavida* deposited eggs on the margin of the leaf, whereas *Z. dolosa* preferentially laid eggs around a caterpillar-infested area or a mechanically wounded spot. *P. pavida* eggs retained their parasitization ability for more than 15 days after they were deposited, whereas the eggs of *Z. dolosa* could not survive more than 5 days after oviposition. Our results suggest that each tachinid parasitoid employs a different host location strategy to exploit semiochemicals coming from plant–herbivore interaction as cues in order to increase their parasitization success.

Keyword HIPVs · Maize · Microtype eggs · Tritrophic interactions · Tachinidae

Introduction

Although the vast majority of parasitoids deposit their eggs directly on or in their hosts, some species oviposit away from their hosts. Some members of the family Tachinidae deposit a large number of very small eggs, termed "microtype" eggs, on the food plants of their herbivorous hosts. The microtype eggs remain dormant on the plant until they are accidentally ingested by a feeding host (Belshaw 1994; Feener and Brown 1997). Such an indirect oviposition strategy may result in a large number of offspring mortality before they are encountered by hosts. To counteract this possibility, microtype tachinids exhibit much higher fecundities (1,000–6,000 eggs) than those of tachinids using the direct oviposition strategy (30–250 eggs; Stireman et al. 2006).

Communicated by: Sven Thatje

R. T. Ichiki (✉) · G. T. T. Ho · S. Nakamura
Japan International Research Center for Agricultural Sciences (JIRCAS),
Ohwashi 1-1,
Tsukuba, Ibaraki 305-8686, Japan
e-mail: richiki22@gmail.com

E. Wajnberg
INRA,
400 Route des Chappes, BP 167, 06903 Sophia Antipolis Cedex, France

Y. Kainoh
Faculty of Life and Environmental Sciences,
University of Tsukuba,
Tennodai 1-1-1,
Tsukuba, Ibaraki 305-8572, Japan

J. Tabata
National Institute for Agro-Environmental Sciences,
Kannondai 3-1-3,
Tsukuba, Ibaraki 305-8604, Japan

In response to herbivory, plants release specific volatile compounds known as herbivore-induced plant volatiles (HIPVs; for review see Turlings and Wäckers 2004; Dicke 2009; Dicke and Baldwin 2010). HIPVs can provide reliable cues indicating the presence of a host, and they have been demonstrated to attract both hymenopteran parasitoids (e.g., Turlings et al. 1990; Vet and Dicke 1992; Potting et al. 1995; Takabayashi et al. 1995; De Moraes et al. 1998; Fukushima et al. 2002; Shiojiri et al. 2006) and several dipteran tachinid parasitoids that use a direct oviposition strategy (Monteith 1955, 1964; Roth et al. 1982; Clement et al. 1986; Kainoh et al. 1999; Ichiki et al. 2008, 2011; Hanyu et al. 2009, 2011). In microtype tachinids, however, the role of HIPVs remains largely unknown.

The microtype tachinids *Pales pavida* (Meigen) (Diptera: Tachinidae) and *Zenillia dolosa* (Meigen) (Diptera: Tachinidae) both belong to the tribe Goniini (Herting and Dely-Draskovits 1993; Tachi and Shima 2010). *P. pavida* occurs throughout the Palearctic region, whereas *Z. dolosa* has been found in Japan and European countries only (Shima 2006). Females of both species lay microtype eggs containing a well-developed first instar larva on the host's food plant, and the eggs ingested with the food hatch inside the host's gut. The tachinid larvae develop by feeding on the bodies of the host and ultimately kill the host. The mature tachinid larvae then emerge from the dead host and pupariate outside. Since *P. pavida* and *Z. dolosa* are polyphagous endoparasitoids that attack a wide range of lepidopteran pests, such as the noctuid moth *Mythimna separata* (Walker) (Lepidoptera: Noctuidae) and the fall webworm *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) (Shima 2006; Ho et al. 2011), they have the potential to control populations of these pests. Understanding the host location behavior of these parasitoids can be useful for manipulating the parasitoid behaviors in order to enhance their impact on pest populations.

Several previous reports have described the biology of *P. pavida* (Huang 1981) and *Z. dolosa* (Ho et al. 2011), but no information on their host location behavior is available. In this study, we evaluated two hypotheses regarding the host location behavior of the microtype tachinids: (1) to increase the probability that hosts encounter their eggs, microtype tachinid females selectively choose suitable plants that contain or are likely to contain hosts, and they deposit their eggs on a particular part of the plant that the herbivorous hosts are likely to eat; and (2) to achieve this, the females may use semiochemicals reflecting host insect–plant interactions as an indication of the presence of their hosts. To test these hypotheses, we investigated the response of *P. pavida* and *Z. dolosa* to plant volatiles, the oviposition site preference and the egg longevity.

Materials and Methods

Insects

M. separata larvae were used as hosts for *P. pavida* and *Z. dolosa*. They were obtained from a stock culture and reared on an artificial diet using the method of Hattori and Atsusawa (1980). The colony of *P. pavida* originated from seven female flies collected at Fukuoka City, Fukuoka, Japan (33°32′N, 130°20′E) in April and May 2005. The colony of *Z. dolosa* originated from 20 individuals that emerged from parasitized final-instar larvae of *H. cunea*, which had been collected at Tsukuba City, Ibaraki, Japan (36°2′N, 140°5′E), in September 2005. The two tachinid species were reared on final-instar (sixth) larvae of *M. separata* as hosts using the method of Ho et al. (2011). Males and females were kept together for mating within 2 days after adult emergence, with each pair in a separate cage (23 cm length, 35 cm width, 20 cm height). Mated females were then transferred to cylindrical plastic cages (8 cm diameter, 14 cm height) with a sugar cube and water and kept alone until the experiments began. Because the preoviposition period is 7 days in *P. pavida* (S. Nakamura, unpublished data) and 6 days in *Z. dolosa* (Ho et al. 2011), females aged 8–18 days were used in the experiments. All experiments were conducted at 25 °C under a photoperiod of 16 h light (L) to 8 h dark (D) and 60 % relative humidity (RH).

Plants

The maize plants (*Zea mays* L., 'Honey-bantam Peter 619', Sakata Seed Co., Kanagawa, Japan) used for wind tunnel and oviposition preference bioassays were grown from seeds sown individually in soil in plastic pots (300 ml) and grown in a greenhouse for more than 1 month (27± 2 °C, 50–70 % RH, 16L:8D). The plants used for the egg longevity experiments were grown from seeds individually sown in soil in plastic cups (50 ml) and grown in an incubator for about 2 weeks (25±1 °C, 50–70 % RH, 16L:8D).

Wind tunnel

The wind tunnel (50 cm width, 150 cm length, 50 cm height) used was the same as the one described by Ichiki et al. (2008). Air was drawn through the tunnel at a speed of 30 cm s^{-1}. Overhead lighting was provided by two fluorescent lights (FLR110H-EX-N/A-100; National Panasonic, Osaka, Japan), and the light intensity on the tunnel floor was 2,600 lx.

Response to plant volatiles

We prepared three kinds of plant samples, caterpillar-infested, mechanically wounded, or intact maize plants for the wind tunnel bioassays. Each maize plant (30–40 cm tall) was cut 5 cm above the ground, and the cut end was placed in an Erlenmeyer flask (200 mL) containing 150 mL of water. The opening of the flask was filled with cotton wool. To make a caterpillar-infested plant sample, ten final-instar larvae of *M. separata* were placed on the cut maize in the flask. After 5 h, we gently removed the larvae and their frass from the plant with a fine brush. In some tachinid parasitoids, frass produced by the host is known to be important for the host location as a kairomonal cue (e.g. Thompson et al. 1983; Clement et al. 1986; Tanaka et al. 2001), although the effects of host frass are currently not surveyed in microtype tachinids. In this study, we focused on effects of plant semiochemicals (synomones) on host location behavior of microtype tachinids and therefore carefully removed host frass from the maize plants in order to eliminate its influence on the tachinid behavior. To make a mechanically wounded plant sample, the leaves of the cut maize in the flask were damaged with a hole punch (5-mm diameter) 30 min before the bioassay. We made 30 holes per plant to damage approximately 20 % of the total surface area of the leaves. To make an intact plant sample, the cut maize in the flask was kept for 5 h after cutting.

No-choice tests were conducted in the wind tunnel. A single intact, mechanically wounded, or caterpillar-infested plant was placed on the upwind side of the wind tunnel. A mated female fly was introduced to the tunnel on a platform (9 cm diameter, 20 cm height) 1 m downwind from the target. A female that did not take off from the platform within 5 min or that did not land on the plant within 5 min after having taken off was regarded as not having been attracted to the target plant and was replaced by a new female. The flight responses to the three target plants were assayed on 11 separate days using a total of 75 *P. pavida* females (25 for each target) and on 8 separate days using a total of 75 *Z. dolosa* females (25 for each target). Each female was tested only once. The target plants were replaced with fresh ones after testing three or four females.

Oviposition site preference

We investigated on which part of a caterpillar-infested or mechanically wounded maize leaf the flies oviposited. A maize leaf was cut to 30 cm in length. To make a caterpillar-infested leaf sample, a final-instar larva of *M. separata* was placed on a cut leaf. The larva was removed after it had eaten one side of the leaf in a semicircular shape approximately 2 cm in diameter. Host frass was also carefully removed from the leaf. To make a mechanically wounded leaf sample, we used scissors to cut one side of a cut leaf in a semicircular shape 2 cm in diameter. The caterpillar-infested or mechanically wounded leaves were used for experiments within 60 min after damage.

A caterpillar-infested or mechanically wounded leaf was placed horizontally on a piece of white paper (30 cm length, 21 cm width) and a clear plastic cage with an open bottom (15 cm length, 11 cm width, 5 cm height) was carefully put over the leaf so that the damaged part was in the center of the cage (Fig. 1). A mated female fly was introduced at the center of the ceiling of the cage, and its behavior was observed for 5 min. We divided the area into four possible oviposition sites: (1) leaf damage (area within 5 mm of the caterpillar-infested or mechanically wounded site); (2) leaf edge (area within 5 mm of the leaf edge except for the damaged part); (3) leaf center (leaf surface except the leaf damage and leaf edge parts): and (4) off of the leaf (paper and cage wall). The number of eggs laid by females at each oviposition site was counted. Oviposition site preference was assayed on ten separate days using 60 *P. pavida* females (31 for caterpillar-infested and 29 for mechanically wounded leaves) and on nine separate days using 63 *Z. dolosa* females (31 for caterpillar-infested and 32 for mechanically wounded leaves) after they started ovipositions. Each female was tested only once, and leaf samples were replaced with fresh ones after every female.

Egg longevity

A potted maize plant (15 cm) was placed in a cage with five to eight mated parasitiod females for oviposition. After 24 h, the plant with eggs was removed from the cage and kept in an incubator (25±1 °C, 50–70 % RH, 16L:8D). *P. pavida* eggs were offered to host larvae for parasitization at 0, 5, 10, 15, and 20 days after oviposition. *Z. dolosa* eggs were offered to host larvae at 0, 5, 10, and 15 days, because eggs of this species were dried up and some had dropped off the plant at 20 days. For this assay, eggs of each species were carefully removed from the plant with a wet brush. Ten eggs were mixed in a piece of artificial diet (50 mg) and offered to a host

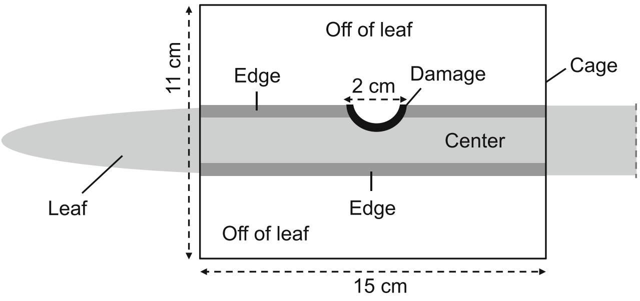

Fig. 1 Top view of the oviposition site preference test set-up, which was divided into four potential oviposition sites: the *leaf damage*, *leaf edge*, *leaf center*, and *off of the leaf*

larva (within 24 h of it having molted to the final instar) in a Petri dish (9 cm diameter, 2 cm height). After 24 h, we checked that the host larva had ingested all eggs. Hosts that did not consume the whole diet or left eggs in the dish were removed from the experiment. Hosts that had ingested eggs were individually reared on an artificial diet in Petri dishes until parasitoids emerged from the hosts for pupariation. The diet was changed daily. When one or more parasitoids emerged from a host, we regarded the eggs as having successfully parasitized the host. For each tachinid species, oviposition was conducted on seven separate days using a total of 50 females. The number of hosts used on each date varied between 38 and 82 due to the availability of caterpillars.

Statistic analysis

The response rates in the wind tunnel assay and parasitism success rates in the egg longevity experiment were analyzed using generalized linear models with a binomial error distribution to assess the effects of treatments. The numbers of eggs deposited at different sites on or near a leaf were analyzed using generalized linear mixed models with a Poisson error distribution to assess the effects of treatments and random effects; this analysis was chosen to account for overdispersion of scores at each of the four possible oviposition sites among the tested females. Calculations were performed by using the function "lmer" of the lme4 package in the software R version 2.7.2 (R Development Core Team 2008). Multiple comparisons were performed with the Benjamini and Hochberg (1995) method, and adjusted *p* values are shown. Finally, a χ^2 test was performed to compare the proportions of females ovipositing on a leaf between the caterpillar-infested and mechanically wounded treatments in the oviposition site preference experiment.

Results

Response to plant volatiles

In the wind tunnel tests, *P. pavida* females responded significantly more to the caterpillar-infested maize plants than to mechanically wounded or intact plants (χ^2=16.7 and 21.8, respectively, *df*=1, *p*<0.001; Fig. 2). *P. pavida* response to mechanically wounded and intact plants was similar (χ^2=0.51, *df*=1, *p*=0.475). On the other hand, the landing rates of *Z. dolosa* were significantly greater on the caterpillar-infested plants than on the intact plants (χ^2=15.4, *df*=1, *p*<0.001) and on the mechanically wounded plants than on the intact plants (χ^2=5.22, *df*=1, *p*=0.022). *Z. dolosa* landing rates on caterpillar-infested and mechanically wounded plants were not significantly different (χ^2= 3.00, *df*=1, *p*=0.083), although slightly more females were landed onto caterpillar-infested plants. There was a significant difference between parasitoid species (χ^2=15.6, *df*=1, *p*<0.001), with *Z. dolosa* showing an overall greater response than *P. pavida*. The overall effect of the plant treatment was also significant (χ^2=40.4, *df*=2, *p*<0.001). No significant interaction was found between the species and plant treatment effects (χ^2=1.67, *df*=1, *p*=0.430).

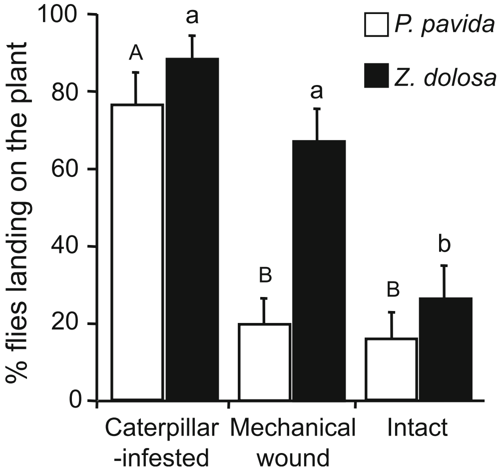

Fig. 2 Flight response (% + SE) of two microtype tachinids to intact, mechanically wounded, and caterpillar-infested maize plants in the wind tunnel. For each plant treatment, 25 females were used for each parasitoid species. *Different uppercase letters* show significant differences between the scores in *Pales pavida* and *different lowercase letters* in *Zenillia dolosa* (χ2, $p < 0.05$)

Oviposition site preference

Of the 31 *P. pavida* females tested on caterpillar-infested leaves, 22 females (71.0 %) oviposited, whereas only 13 of 29 females (44.8 %) oviposited on the mechanically wounded leaves (χ^2=4.21, *df*=1, *p*=0.04). When *P. pavida* females contacted a leaf, they began to walk around the leaf while tapping the surface with their legs. Once they reached the edge, they grasped it with their front tarsi, extended the ovipositor, and attached an egg by the edge of leaf. *P. pavida* females showed a significant oviposition preference for the edge of caterpillar-infested (χ^2=508.8, *df*=3, *p*<0.001) and mechanically wounded (χ^2=182.9, *df*=3, *p*<0.001) leaves (Fig. 3a). Moreover, there was a significant effect of plant treatment (χ^2=12.2, *df*=1, *p*<0.001), with more eggs laid by *P. pavida* on caterpillar-infested than on mechanically wounded leaves.

Twenty-six of the 31 (83.9 %) *Z. dolosa* females oviposited on caterpillar-infested leaves, and 25 of 32 (78.1 %) oviposited on mechanically wounded leaves (χ^2=0.337, *df*=1, *p*=0.561). When *Z. dolosa* females touched the damaged site of a leaf, they intensively tapped the damaged site with their front tarsi, extended their ovipositor, and attached

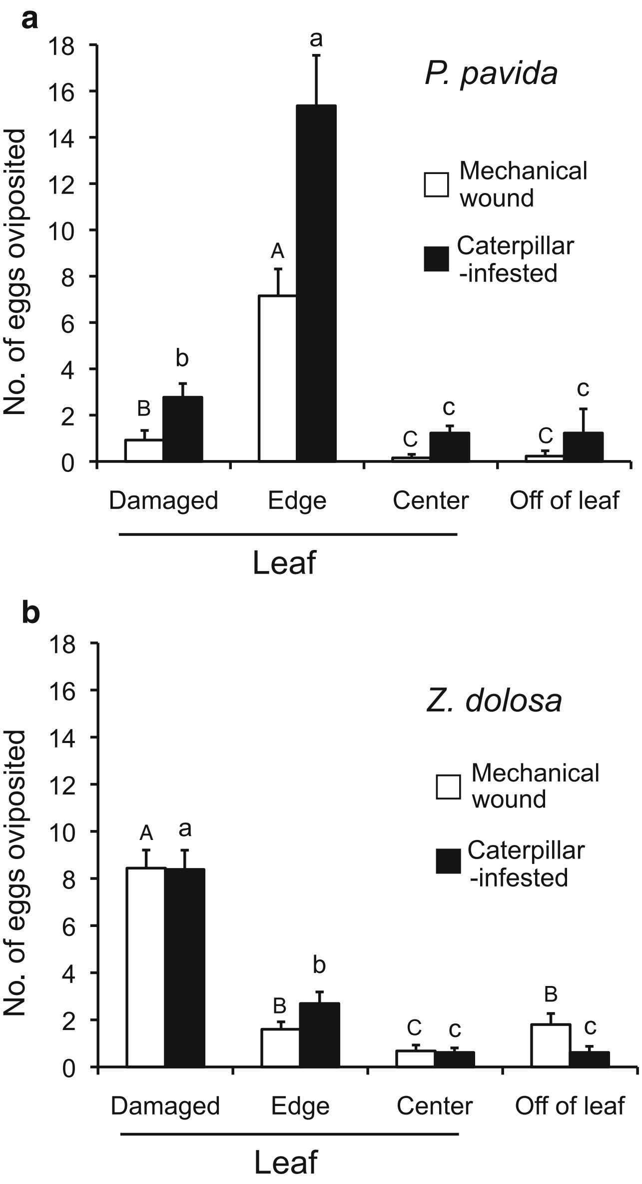

Fig. 3 Mean number of eggs (+SE) deposited by *Pales pavida* (**a**) and *Zenillia dolosa* (**b**) at different sites on mechanically wounded or caterpillar-infested maize leaves. *Different uppercase letters* show significant differences between the scores in mechanically wounded leaves and *different lowercase letters* in caterpillar-infested leaves ($\chi 2$, $p < 0.05$)

an egg beside the damaged site. The females then continued to explore and oviposit near the damaged site. *Z. dolosa* females laid significantly more eggs at the damaged site on both caterpillar-infested (χ^2=315.4, *df*=3, p<0.001) and mechanically wounded leaves (χ^2=263.2, *df*=3, p<0.001; Fig. 3b). However, no significant difference was found in the numbers of eggs laid between the two plant treatments (χ^2=0.0049, *df*=1, p=0.944).

Our analysis revealed a significant interaction between the species and the preferred location on both caterpillar-infested (χ^2=268.0, *df*=3, p<0.001) and mechanically wounded leaves (χ^2=193.5, *df*=3, p<0.001), indicating that the two species chose different locations on maize leaves to lay eggs.

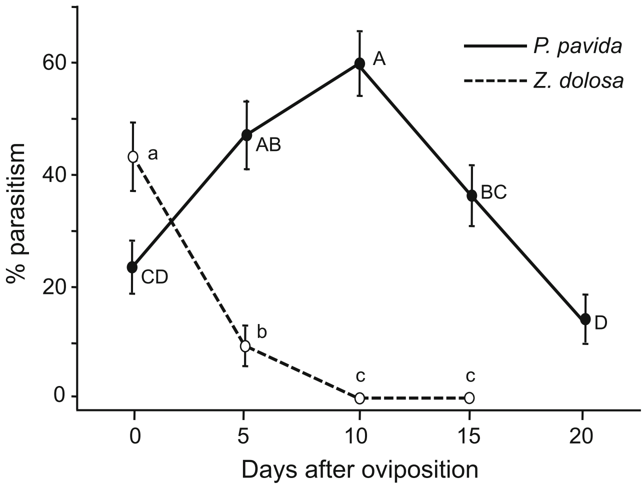

Fig. 4 Daily fluctuation of parasitization success (% ± SE) of eggs after 25 being deposited on maize leaves. *Different uppercase letters* show significant differences between the scores in *Pales pavida* and *different lowercase letters* in *Zenillia dolosa* ($\chi 2$, $p < 0.05$)

Egg longevity

In *P. pavida*, the parasitization success of eggs peaked at 10 days after oviposition (reaching 59.5 %) and then decreased (χ^2=42.6, *df*=4, p<0.001; Fig. 4). However, the eggs' parasitization ability remained substantial (>10 %) even at 20 days after oviposition. In *Z. dolosa*, however, the parasitization success of eggs was highest just after oviposition (43.5 %) and then rapidly decreased (χ^2=63.9, *df*=3, p<0.001). *Z. dolosa* eggs completely lost their parasitization ability by 10 days after oviposition. A significant interaction was found between species and egg longevity (χ^2=81.4, *df*=3, p<0.001), which means that daily fluctuations in the parasitization success of eggs after oviposition differed between the two species.

Discussion

Our results demonstrate that the microtype tachinids *P. pavida* and *Z. dolosa* use different oviposition strategies. *P. pavida* females were significantly more attracted to volatiles emitted from maize plants infested by *M. separata* larvae and preferentially laid their eggs on the edge of maize leaves. The eggs survived for more than 15 days after oviposition. On the other hand, *Z. dolosa* females were attracted to volatiles from both caterpillar-infested and mechanically wounded maize plants and concentrated their egg-laying around a caterpillar-infested area or a mechanically wounded spot. Most of the eggs died within 5 days.

Their attraction to *M. separata*-infested maize plants indicates that *P. pavida* females perceive specific HIPVs released from plants in response to herbivory by caterpillars. Attraction to HIPVs has been reported in many parasitoid species (for review, see Turlings and Wäckers 2004; Dicke 2009; Dicke and Baldwin 2010), but in only three species of microtype tachinids: *Cyzenis albicans* (Fallén) was attracted to volatiles from oak trees infested by the winter moth *Operophtera brumata* (L.) (Lepidoptera: Geometridae) (Roland et al. 1989, 1995), and both *Leschenaultia exul* (Townsend) and *Patelloa pachypyga* (Aldrich and Webber) were attracted to volatiles from aspen trees infested by the forest tent caterpillar *Malacosoma disstria* (Hübner) (Lepidoptera: Lasiocampidae) (Mondor and Roland 1997). Takabayashi et al. (1995) reported that maize plants infested by final-instar larvae of *M. separata* release several HIPVs, including (*E*)-4,8-dimethyl-1,3,7-nonatriene and indole. Another study found that emission of these HIPVs starts about 1 h after herbivory and continues for more than 12 h after the initial damage (Turlings et al. 1998). In addition, HIPVs were shown to be released systemically in large amounts throughout the plant (Turlings and Tumlinson 1992). Our results suggest that *P. pavida* can use HIPVs as cues to locate a suitable plant where their hosts are likely to be found.

Our results suggest that the HIPVs not only attract *P. pavida*, but also elicit oviposition. Significantly more *P. pavida* females oviposited on caterpillar-infested maize leaves than on mechanically wounded leaves, and significantly more eggs were laid on caterpillar-infested leaves compared to mechanically wounded leaves. The use of HIPVs as oviposition stimuli may induce *P. pavida* females to deposit their eggs over a much wider area, because these volatiles have a high volatility and are known to be commonly detected by parasitoids from a distance (Vet and Dicke 1992). Our results show that *P. pavida* eggs can wait for hosts for at least 15 days after they are deposited on leaves. Furthermore, we found that fresh eggs of *P. pavida* had lower parasitization ability than older eggs, suggesting that it is not advantageous for the eggs to be ingested by hosts soon after they have been oviposited. This result is consistent with a report by Huang (1981), who found that at 23 °C the egg hatchability of *P. pavida* was about 70 % 1 day after oviposition, but increased to about 90 % after 1 week. We also found that *P. pavida* females preferred to lay their eggs along the edge of maize leaves. Hirai and Ishii (2002) demonstrated that there is a close relationship between the oviposition site preference of the microtype tachinid *Sturmia bella* (Meigen) and the feeding behavior of its host *Parantica sita* (Koller) (Lepidoptera: Danaidae). The fourth- and fifth-instar host larvae, which are of suitable age for parasitization by *S. bella*, ate the marginal or apical area of leaves of the evergreen milkvine, where the density of the tachinid eggs was high. The preference of *P. pavida* to oviposit on the leaf edge likely increases the probability that hosts encounter the eggs, because the fifth and sixth instars of *M. separata*, which are of suitable age for parasitization by *P. pavida*, always start feeding on a maize leaf from the edge (R.T. Ichiki, personal observation).

Z. dolosa females were attracted by volatiles from mechanically wounded plants, suggesting that the species responds to green leaf volatiles (GLVs), which are commonly released from plants after physical damage as well as by herbivory (e.g., Takabayashi et al. 1995; Turlings et al. 1998; Hoballah et al. 2002; Chamberlain et al. 2006) and are known to attract parasitic wasps such as *Cotesia* (=*Apanteles*) *kariyai* (Watanabe) (Hymenoptera: Braconidae) (Takabayashi et al. 1991) and *Aphidius rhopalosiphi* Dalman (Hymenoptera: Aphelinidae) (Wickremasinghe and van Emden 1992). Turlings et al. (1998) reported that immediately after damage, maize plants release large amounts of GLVs such as (*E*)-2-hexenal, (*Z*)-3-hexenol, and (*Z*)-3-hexenyl acetate, and the amounts released drop rapidly after the first hour. *Z. dolosa* females could use these short-lived volatiles as cues to pinpoint the location where their hosts were recently feeding. Our results show that *Z. dolosa* females preferentially deposited their eggs near the areas damaged by feeding hosts or mechanical wounding. Moreover, eggs of *Z. dolosa* survived only a few days after oviposition, indicating that eggs need to be ingested by host larvae soon after oviposition. Laying eggs close to the host-feeding area would increase the probability that *Z. dolosa* eggs are ingested by their hosts.

The fact that *Z. dolosa* females concentrate their oviposition around areas of host infestation or mechanical wounds implies that contact chemicals from plant leaf exudates may be important for oviposition in this tachinid species. Plant leaf exudates have been shown to elicit oviposition in other microtype tachinids. For example, when females of the tachinid *Blepharipa pratensis* (Meigen) contact a recently damaged plant edge, they orient perpendicular to the edge and move back and forth with their front tarsi grasping the damaged edge (Odell and Godwin 1984). Leaf exudates arrest females on the leaf and increase tarsal examination behavior. If they contact the edge of a damaged leaf, oviposition usually follows. We observed that *Z. dolosa* females have a similar tarsal examination behavior on the damaged area of maize leaves. Tarsal contact with damaged leaves has also been shown in the oviposition behavior of two other microtype tachinids, *C. albicans* (Hassell 1968; Roland 1986) and *L. exul* (Mondor and Roland 1997). Hassell (1968) demonstrated that sugars at the edge of feeding-damaged oak leaves act as an oviposition stimulant for *C. albicans*.

The present study suggests that these two microtype tachinid species can effectively search host habitat by using different semiochemicals derived from plants infested by their host caterpillars. *P. pavida* uses HIPVs as cues to locate the plants that contain potential hosts, whereas *Z. dolosa* uses GLVs as cues to pinpoint the location where their hosts were recently feeding. We also found a clear difference in the oviposition site preference between the two species, which appears to be related to longevity of their eggs. *P. pavida* lays eggs on the edge of leaves, close to where hosts are, but their eggs are able to wait for hosts for more than 15 days after oviposition. On the other hand, *Z. dolosa* concentrates its eggs close to an area of host infestation, but the eggs die within a few days after oviposition. To increase the rate of parasitization success, it is possible that each microtype tachinid has evolved a host location strategy that is closely related to a particular type of semiochemical coming from plant–herbivore interactions.

Acknowledgments We are grateful to Drs. Hiroshi Shima and Takuji Tachi of Kyushu University for their useful comments on the manuscript and for providing insect materials. We also thank Aki Konishi, Yuriko Akagi, Yukie Muraki, and Chieko Yokokura of the Japan International Research Center for Agricultural Sciences for technical assistance. This work was supported by a Research Fellowship (to Ryoko T. Ichiki) through the Japan Society for the Promotion of Science.

References

Belshaw R (1994) Life history characteristics of Tachinidae (Diptera) and their effect on polyphagy. In: Hawkins BA, Sheehan W (eds) Parasitoid community ecology. Oxford University Press, New York, pp 145–162

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Roy Statist Soc Ser B 57:289–300

Chamberlain K, Khan ZR, Pickett JA, Toshova T, Wadhams LJ (2006) Diel periodicity in the production of green leaf volatiles by wild and cultivated host plants of stemborer moths, *Chilo partellus* and *Busseola fusca*. J Chem Ecol 32:565–577

Clement SL, Rubink WL, McCartney DA (1986) Larviposition response of *Bonnetia comta* (Dipt.: Tachinidae) to a kairomone of *Agrotis ipsilon* (Lep.: Noctuidae). Entomophaga 31:277–284

De Moraes CM, Lewis WJ, Paré PW, Alborn HT, Tumlinson JH (1998) Herbivore-infested plants selectively attract parasitoids. Nature 393:570–573

Dicke M (2009) Behavioural and community ecology of plants that cry for help. Plant Cell Environ 32:654–665

Dicke M, Baldwin IT (2010) The evolutionary context for herbivore-induced plant volatiles: beyond the ‘cry for help’. Trends Plant Sci 15:167–175

Feener DH Jr, Brown BV (1997) Diptera as parasitoids. Annu Rev Entomol 42:73–97

Fukushima J, Kainoh Y, Honda H, Takabayashi J (2002) Learning of herbivore-induced and nonspecific plant volatiles by a parasitoid, *Cotesia kariyai*. J Chem Ecol 28:579–586

Hanyu K, Ichiki RT, Nakamura S, Kainoh Y (2009) Duration and location of attraction to herbivore-damaged plants in the tachinid parasitoid *Exorista japonica*. Appl Entomol Zool 44:371–378

Hanyu K, Ichiki RT, Nakamura S, Kainoh Y (2011) Behavior of the tachinid parasitoid *Exorista japonica* (Diptera: Tachinidae) on herbivore-infested plants. Appl Entomol Zool 46:565–571

Hassell MP (1968) The behavioural response of a tachinid fly (*Cyzenis albicans* (Fall.)) to its host, the winter moth (*Operophtera brumata* (L.)). J Anim Ecol 37:627–639

Hattori M, Atsusawa S (1980) Mass-rearing of the cabbage armyworm, *Mamestra brassicae* Linné, and the common armyworm, *Mythimna separata* Walker (Lepidoptera: Noctuidae), on a simple artificial diet. Jpn J Appl Entomol Zool 24:36–38 (in Japanese with English summary)

Herting B, Dely-Draskovits A (1993) Family Tachinidae. In: Soós Á, Papp L (eds) Catalogue of Palaearctic Diptera, vol 13, Anthomyiidae–Tachinidae. Hungarian Natural History Museum, Budapest, pp 118–458

Hirai N, Ishii M (2002) Egg placement of the tachinid fly *Sturmia bella* on leaves of the evergreen milkvine *Marsdenia tomentosa* and the feeding habit of its host butterfly *Parantica sita*. Entomol Sci 5:153–159

Ho GTT, Ichiki RT, Nakamura S (2011) Reproductive biology of the microtype tachinid fly *Zenillia dolosa* (Meigen) (Diptera: Tachinidae). Entomol Sci 14:210–215

Hoballah MEF, Tamo C, Turlings TCJ (2002) Differential attractiveness of induced odors emitted by eight maize varieties for the parasitoid *Cotesia marginiventris*: Is quality of quantity important? J Chem Ecol 28:951–962

Huang P (1981) Laboratory rearing of *Pales pavida* (Dip: Tachinidae) on substitute host *Galleria mellonella* (Lep: Galleriidae). Z Pflanzenk Pflanzen 88:177–188 (in German with English summary)

Ichiki RT, Kainoh Y, Kugimiya S, Takabayashi J, Nakamura S (2008) Attraction to herbivore-induced plant volatiles by the host-foraging parasitoid fly *Exorista japonica*. J Chem Ecol 34:614–621

Ichiki RT, Kainoh Y, Yamawaki Y, Nakamura S (2011) The parasitoid fly *Exorista japonica* uses visual and olfactory cues to locate herbivore-infested plants. Entomol Exp Appl 138:175–183

Kainoh Y, Tanaka C, Nakamura S (1999) Odor from herbivore-damaged plant attracts a parasitoid fly, *Exorista japonica* Townsend (Diptera: Tachinidae). Appl Entomol Zool 34:463–467

Mondor EB, Roland J (1997) Host locating behaviour of *Leschenaultia exul* and *Patelloa pachypyga*: two tachinid parasitoids of the forest tent caterpillar, *Malacosoma disstria*. Entomol Exp Appl 85:161–168

Monteith LG (1955) Host preferences of *Drino bohemica* Mesn. (Diptera: Tachinidae), with particular reference to olfactory responses. Can Entomol 87:509–530

Monteith LG (1964) Influence of the health of the food plant of the host on host-finding by tachinid parasites. Can Entomol 96:1477–1482

Odell TM, Godwin PA (1984) Host selection by *Blepharipa pratensis* (Meigen), a tachinid parasite of the gypsy moth, *Lymantria dispar* L. J Chem Ecol 10:311–320

Potting RPJ, Vet LEM, Dicke M (1995) Host microhabitat location by stem-borer parasitoid *Cotesia flavipes*: the role of herbivore volatiles and locally and systemically induced plant volatiles. J Chem Ecol 21:525–539

R Development Core Team (2008) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available via http://www.R-project.org

Roland J (1986) Parasitism of winter moth in British Columbia during build-up of its parasitoid *Cyzenis albicans*: attack rate on oak v. apple. J Anim Ecol 55:215–234

Roland J, Evans WG, Myers JH (1989) Manipulation of oviposition patterns of the parasitoid *Cyzenis albicans* (Tachinidae) in the field using plant extracts. J Insect Behav 2:487–503

Roland J, Denford KE, Jimenez L (1995) Borneol as an attractant for *Cyzenis albicans*, a tachinid parasitoid of the winter moth, *Operophtera brumata* L. (Lepidoptera, Geometridae). Can Entomol 127:413–421

Roth JP, King EG, Hensley SD (1982) Plant, host, and parasite interactions in the host selection sequence of the tachinid *Lixophaga diatraeae*. Environ Entomol 11:273–277

Shima H (2006) A host–parasite catalog of Tachinidae (Diptera) of Japan. Makunagi/Acta Dipt 31:1–171

Shiojiri K, Ozawa R, Matsui K, Kishimoto K, Kugimiya S, Takabayashi J (2006) Role of the lipoxygenase/lyase pathway of host–food plants in the host searching behavior of two parasitoid species, *Cotesia glomerata* and *Cotesia plutellae*. J Chem Ecol 32:969–979

Stireman JO III, O'Hara JE, Wood DM (2006) Tachinidae: evolution, behavior, and ecology. Annu Rev Entomol 51:525–555

Tachi T, Shima H (2010) Molecular phylogeny of the subfamily Exoristinae (Diptera, Tachinidae), with discussions on the evolutionary history of female oviposition strategy. Syst Entomol 35:148–163

Takabayashi J, Noda T, Takahashi S (1991) Plants produce attractants for *Apanteles kariyai*, a parasitoid of *Pseudaletia separata*; cases of 'communication' and 'misunderstanding' in parasitoid–plant interactions. Appl Entomol Zool 26:237–243

Takabayashi J, Takahashi S, Dicke M, Posthumus MA (1995) Developmental stage of the herbivore *Pseudaletia separata* affects production of herbivore-induced synomone by maize plants. J Chem Ecol 21:273–287

Tanaka C, Kainoh T, Honda H (2001) Host frass as arrestant chemicals in locating host *Mythimna separata* by the tachinid fly *Exorista japonica*. Entomol Exp Appl 100:173–178

Thompson AC, Roth JP, King EG (1983) Larviposition kairomone of the tachinid *Lixophaga diatraeae*. Environ Entomol 12:1312–1314

Turlings TCJ, Tumlinson JH (1992) Systemic release of chemical signals by herbivore-injured maize. Proc Natl Acad Sci USA 89:8399–8402

Turlings TCJ, Wäckers FL (2004) Recruitment of predators and parasitoids by herbivore-injured plants. In: Cardé RT, Millar JG (eds) Advances in insect chemical ecology. Cambridge University Press, Cambridge, pp 21–75

Turlings TCJ, Tumlinson JH, Lewis WJ (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. Science 250:1251–1253

Turlings TCJ, Lengwiler UB, Bernasconi ML, Wechsler D (1998) Timing of induced volatile emissions in maize seedlings. Planta 207:146–152

Vet LEM, Dicke M (1992) Ecology of infochemical use by natural enemies in a tritrophic context. Annu Rev Entomol 37:141–172

Wickremasinghe MGV, van Emden HF (1992) Reactions of adult female parasitoids, particularly *Aphidius rhopalosiphi*, to volatile chemical cues from the host plants of their aphid prey. Physiol Entomol 17:297–304